BRCA Gene Mutation Analysis using NGS

DNA

Diligence N Ability

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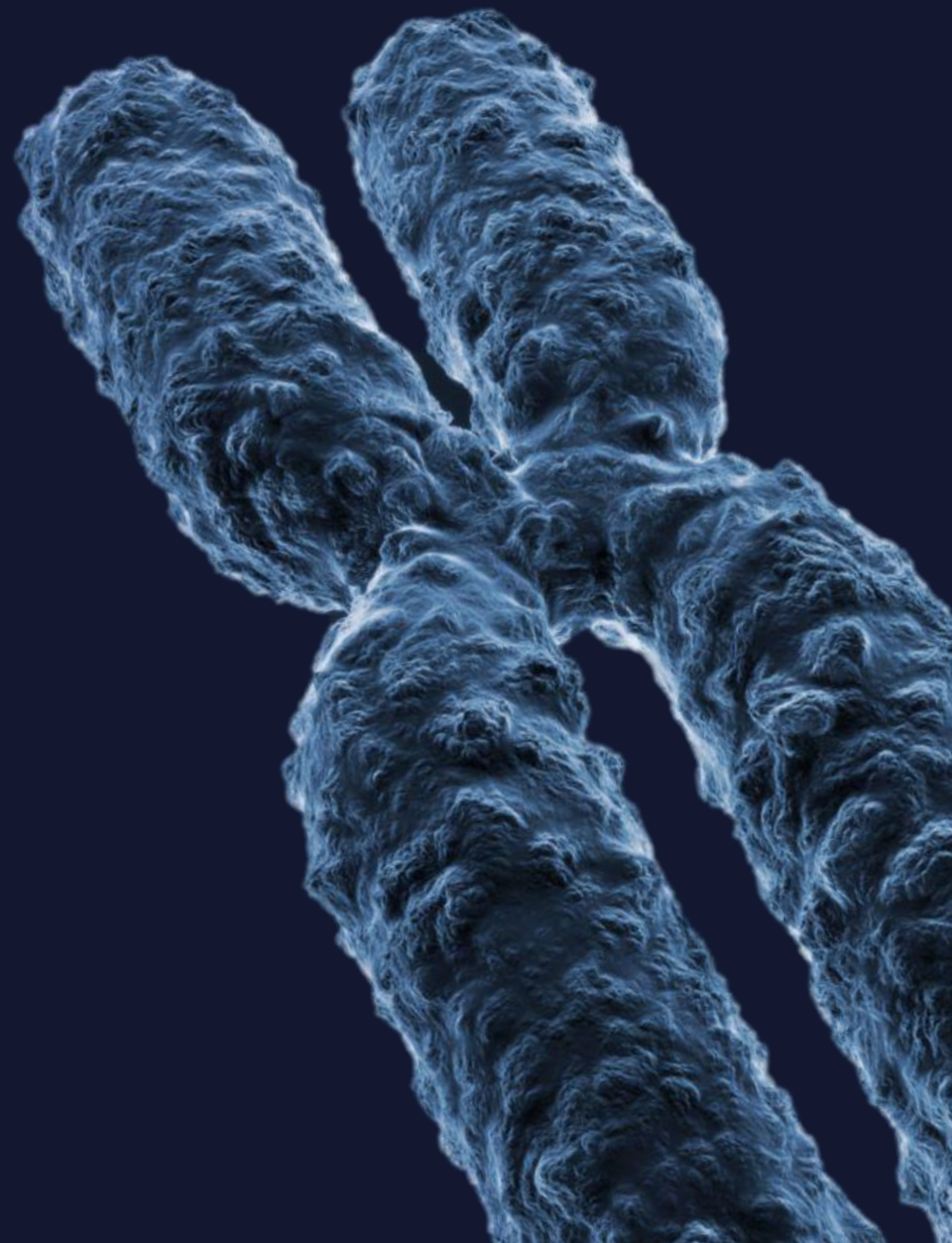
2 학 년 : 김혜숙, 박민정, 김다해, 정지연, 김다은, 김민서, 김지영
1 학 년 : 강희정, 김동화, 김민철, 김예담, 김은지, 노은영
민문경, 이민제, 변은빈, 오승헌, 최수진, 추효림



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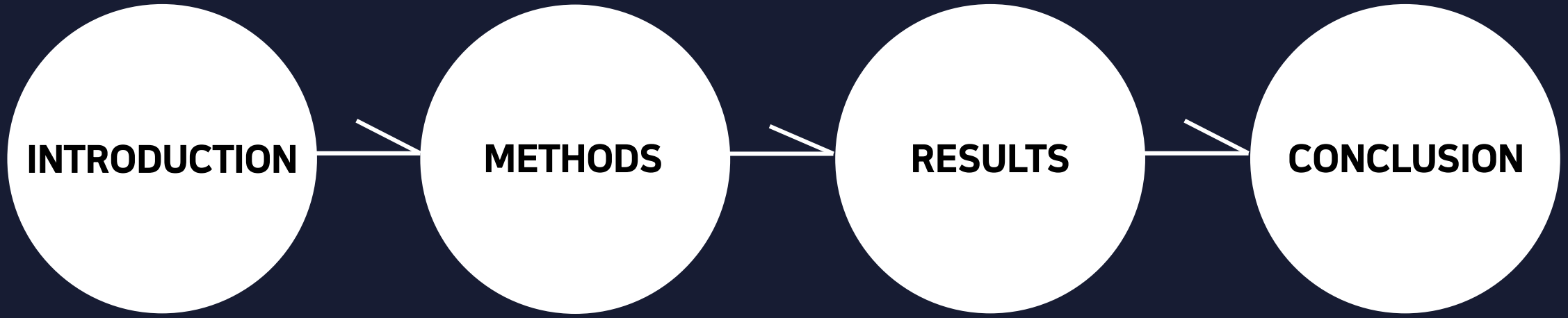


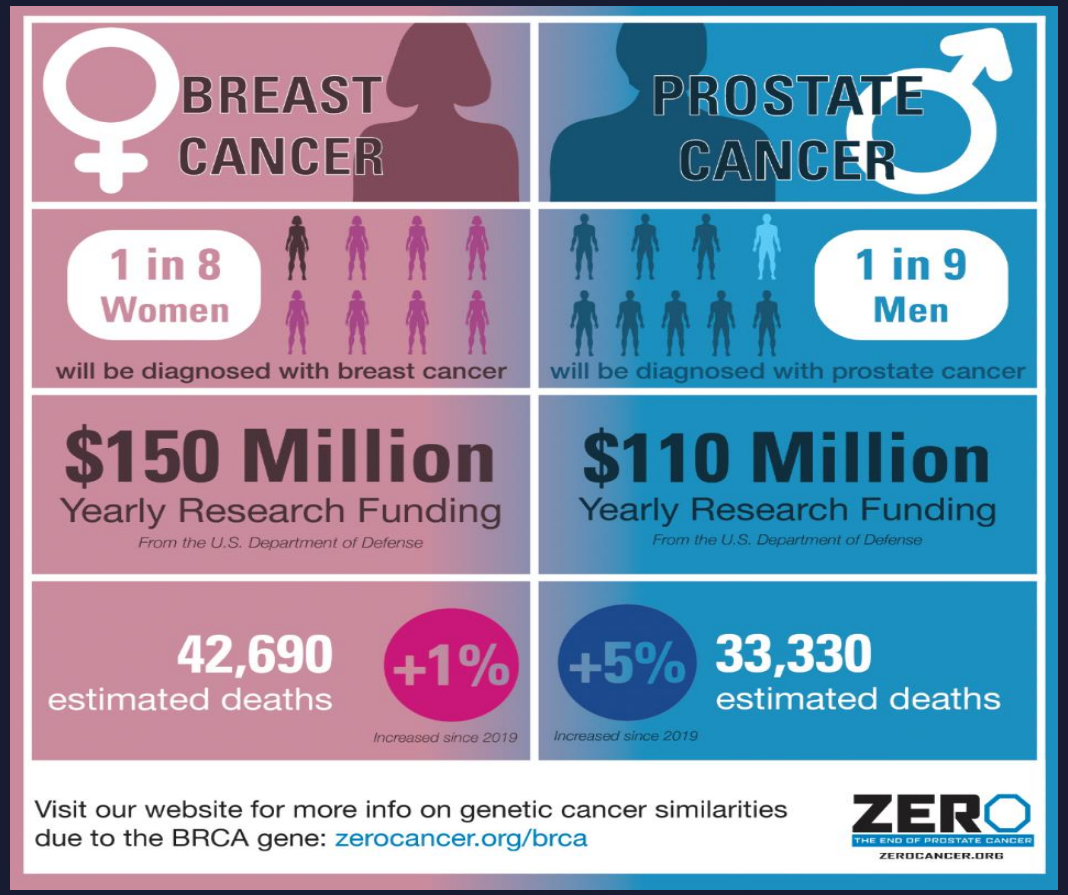
The incidence of breast cancer in Korea
Advances in breast cancer diagnosis

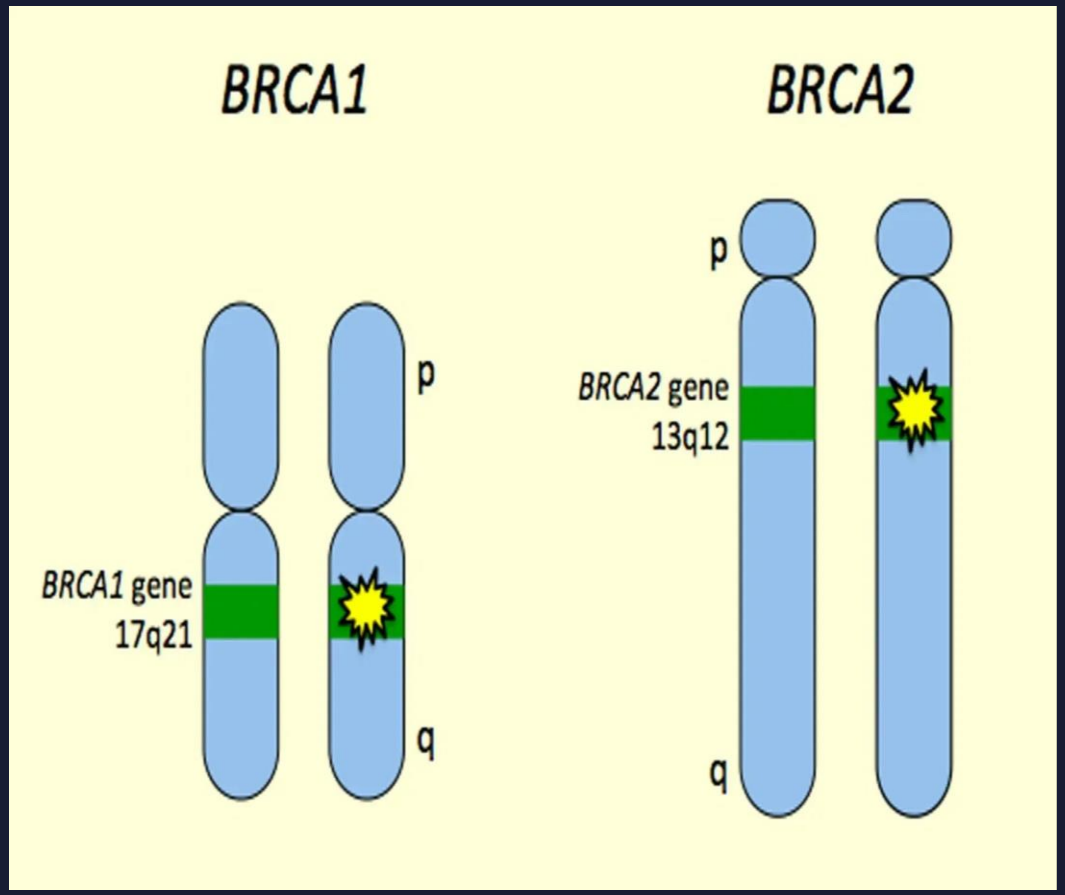
HEMEACCUTEST
for BRCA mutation test

Dentification of gene mutations
using NGS panels

Development of NGS in the field of
molecular diagnostic medicine.

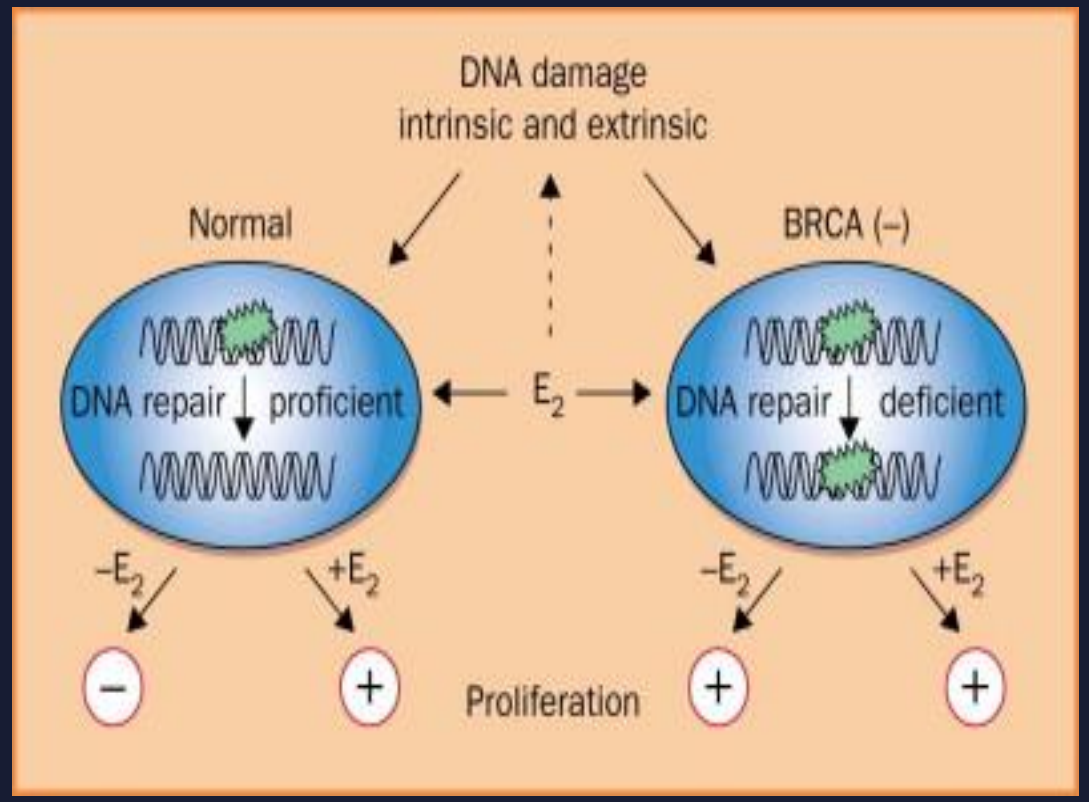
INTRODUCTION

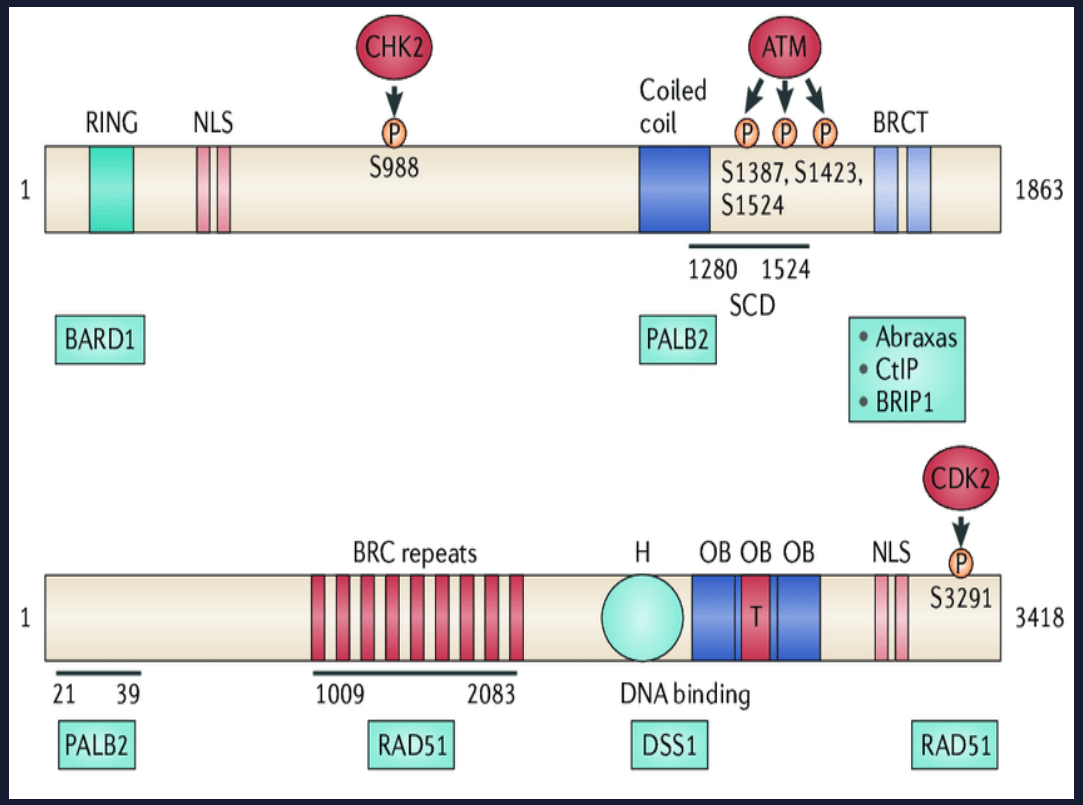




Breast cancer is the most common type of cancer in women worldwide, and nearly 12,000 breast cancer patients occur every year in Korea. In fact, according to domestic statistics, the incidence of breast cancer in women from 2001 to 2018 is increasing by 6.6% annually.

The BRCA (BReast CAncer) gene is known to be the most associated with hereditary breast cancer,
About 15-40% of hereditary breast cancer is caused by pathogenic mutations (PVs) in BRCA1 and BRCA2.





The BRCA1 gene is located in chromosome 17q21, has 24 exons, has 1863 amino acids, BRCA2 is composed of 27 exons, has 3,418 amino acids, and the BRCA gene does not produce abnormal protein and undergo DNA damage.

암 유발 변이 유전자 찾는 'RNA패널' 검사법 개발

폐암·뇌종양·육종 등 고형암에서 융합유전자변이 검출 우수

'NGS검사'…돌연변이 유전자 찾아내 유방·난소암 억제

유전성 암, 'NGS검사'로 조기발견·치료율 높인다

혈액검사로 20여종 암 진단하는 시대 열린다

클리노믹스, 액체생검 기반의 miRNA진단법 개발로 '암진단'

NGS

Next Generation Sequencing

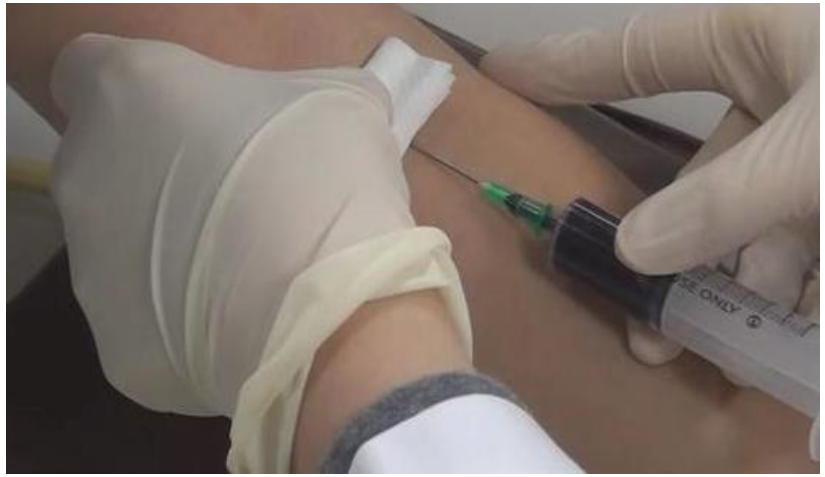
PURPOSE OF THE STUDY

This study attempted to analyze BRCA gene mutations in 10 clinical pathology students at Daejeon Health Science University using NGS panel tests, which are widely used in the diagnosis of various cancer diseases, and to compare and analyze differences from conventional mutation analysis methods.

METHODS

10 SUBJECTS

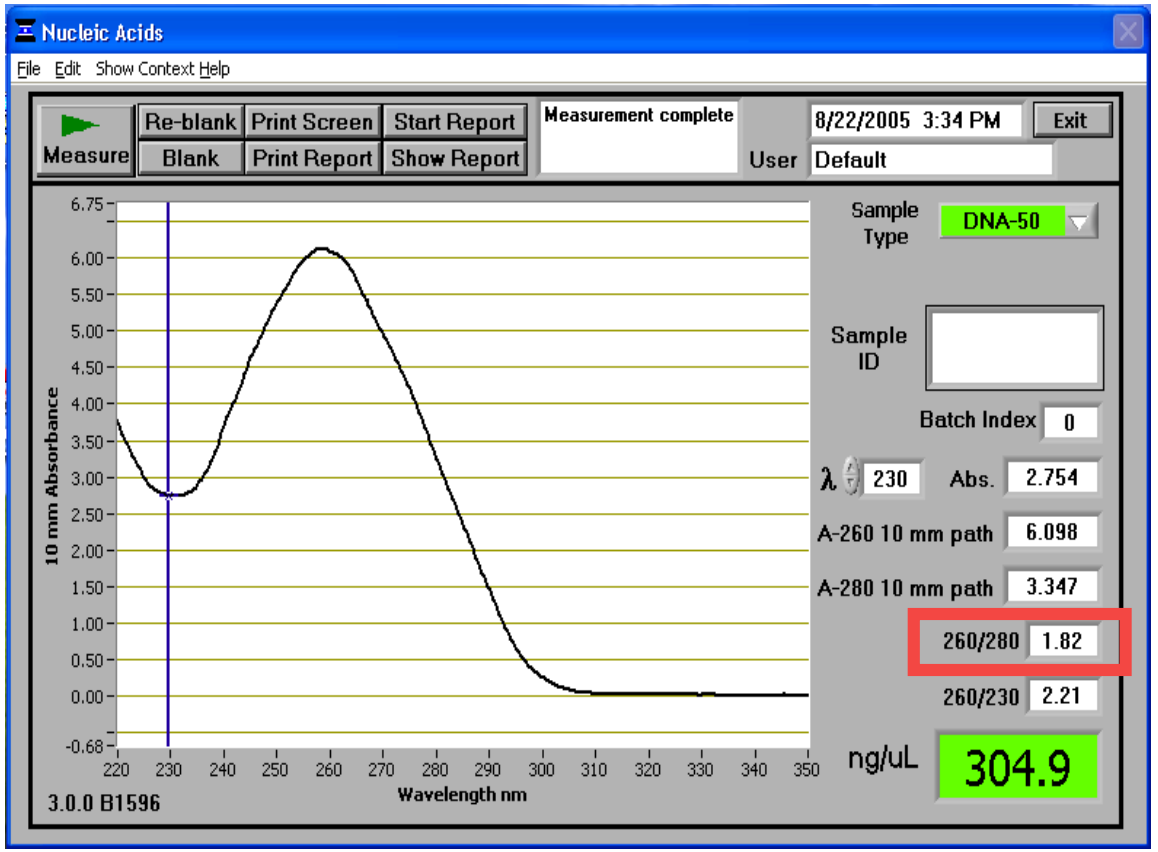
DNA Extration



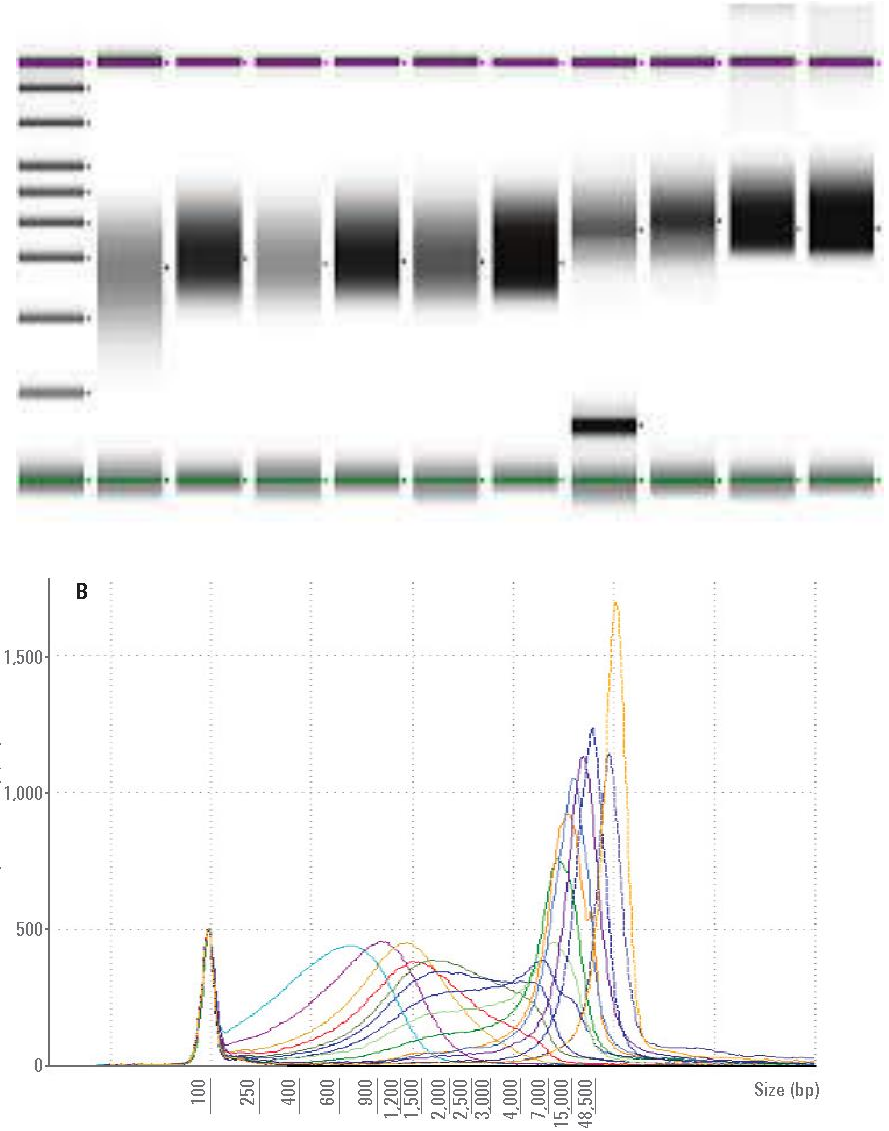
Pre-treatment

Genomic DNA Extration

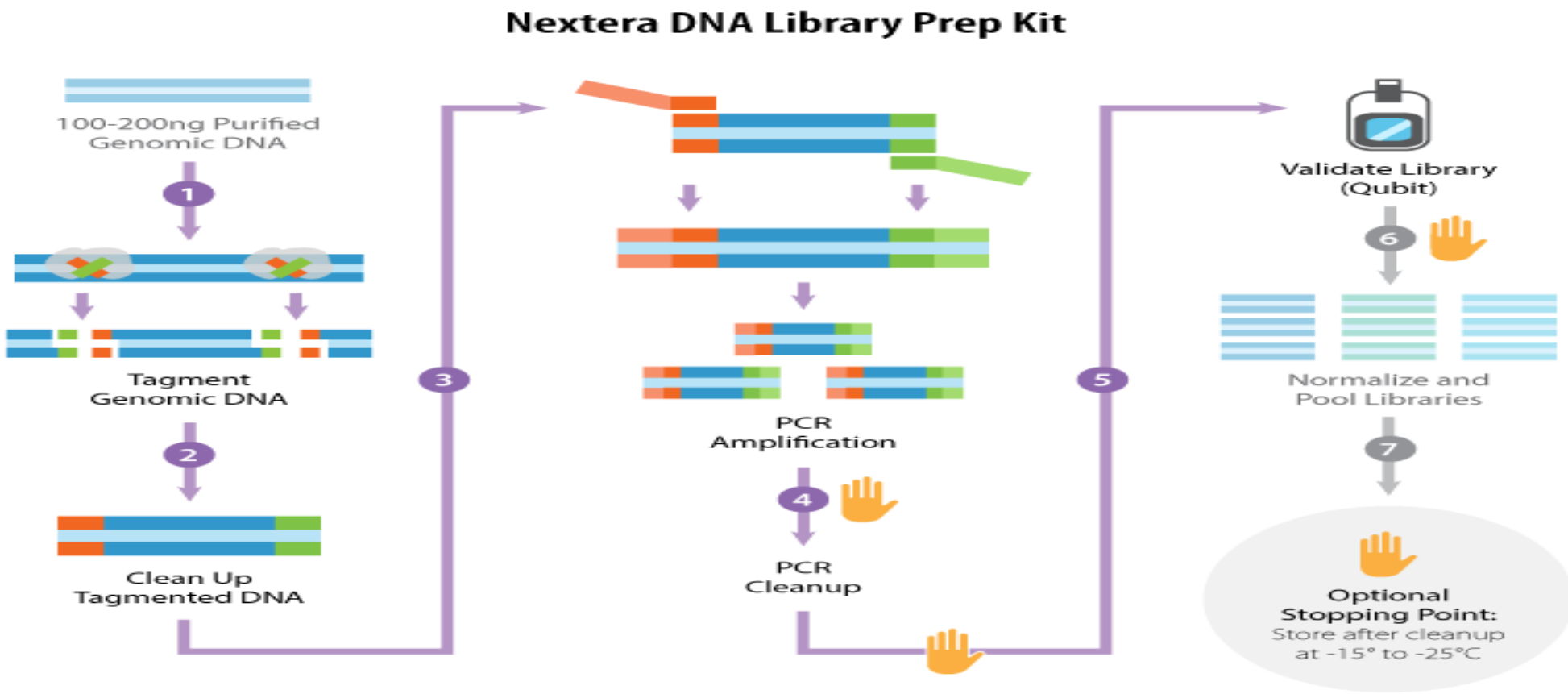
DNA Quantitation



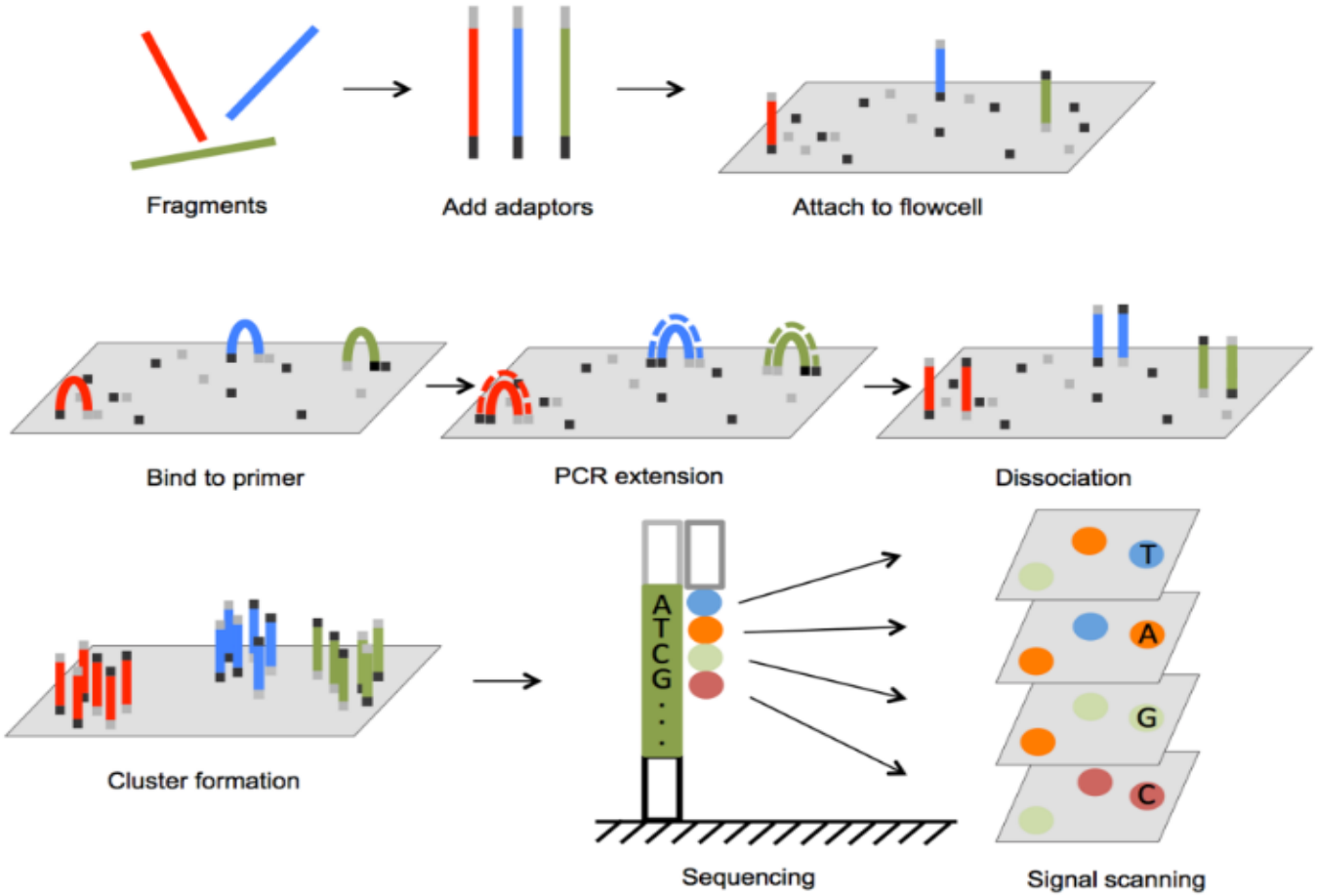
DNA Quantitation



Library Construction



NGS using MiSeq



Mutant Analysis

NGeneBio

Local Server or

Cloud Server

Visualization %

Clinical reports generation

NETWORK

Upload raw data files

NGeneAnalysis

Client tools

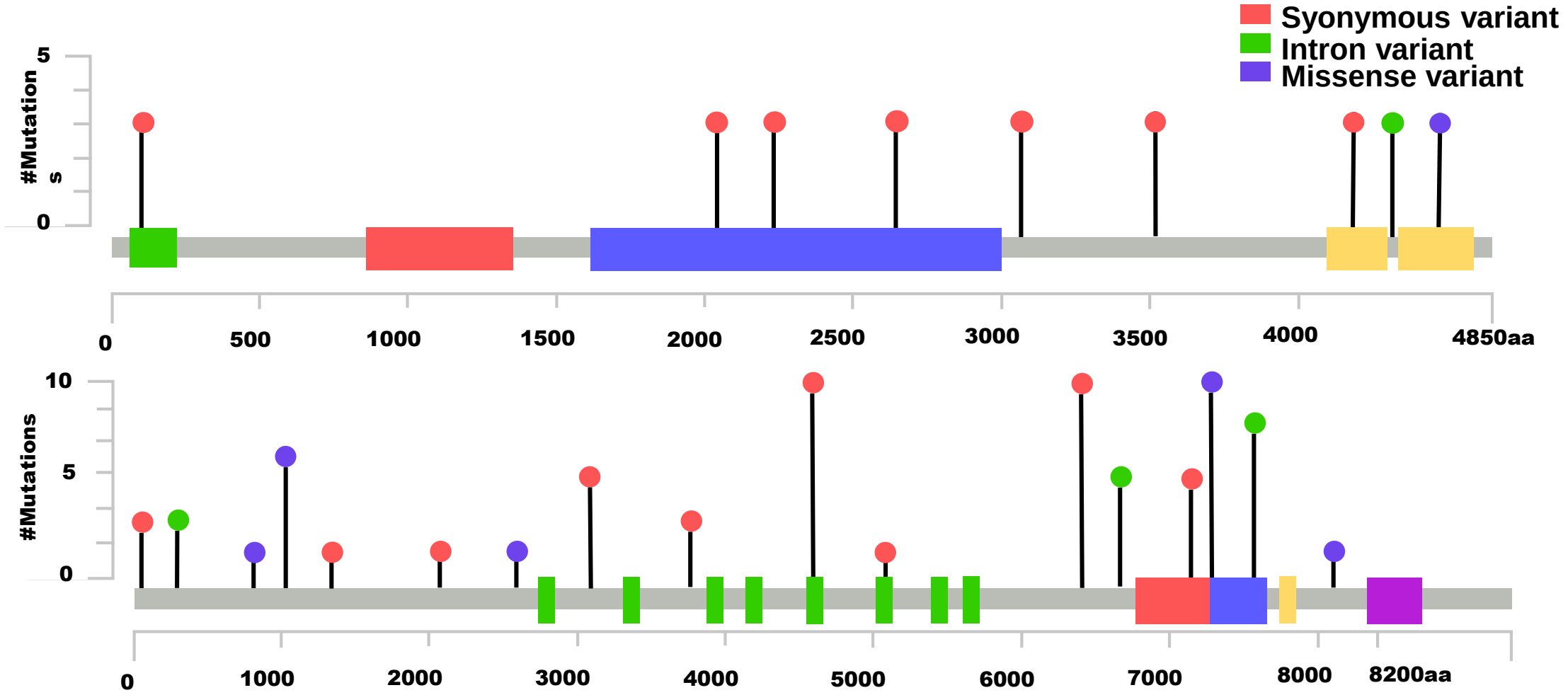
RESULTS

Variants found through genetic testing are currently classified and reported as follows:

Pathogenic Mutation	Alterations with sufficient evidence to classify as pathogenic (capable of causing disease). Targeted testing of at-risk family members and appropriate changes in medical management (i.e. high risk surveillance) for pathogenic mutation carriers recommended. A pathogenic mutation is always included in results reports.
Variant, Likely Pathogenic (VLP)	Alterations with strong evidence in favor of pathogenicity. Targeted testing of at-risk family members and appropriate changes in medical management (i.e. high risk surveillance) for VLP carriers recommended. A VLP is always included in results reports.
Variant, Unknown Significance (VUS)	Alterations with limited and/or conflicting evidence regarding pathogenicity. Targeted testing of informative family members to collect cosegregation data via our Family Studies Program recommended. Medical management based on personal and family histories, not VUS carrier status. A VUS is always included in results reports.
Variant, Likely Benign (VLB)	Alterations with strong evidence against pathogenicity Targeted testing of at-risk family members not recommended. Medical management based on personal and family histories. A VLB is not routinely included in results reports.
Benign	Alterations with very strong evidence against pathogenicity. Targeted testing of at-risk family members not recommended. Medical management based on personal and family histories. Benign alterations are not routinely included in results reports.

SUBJECTS	BRCA1	Zygosity	Type	BRCA2	Zygosity	Type
SUB 1				c.7806-14T>C	hetero	snv
				c.7397T>C	hetero	snv
				c.6513G>C	hetero	snv
				c.4563A>G	hetero	snv
				c.3807T>C	hetero	snv
				c.2971A>G	hetero	snv
				c.2229T>C	hetero	snv
				c.1365A>G	hetero	snv
				c.865A>C	hetero	snv
				c.425+67A>C	hetero	snv
SUB 2				c.7806-14T>C	hetero	snv
				c.7397T>C	hetero	snv
				c.7242A>G	hetero	snv
				c.6841+80_6841+83delTTAA	hetero	del
				c.6513G>C	hetero	snv
				c.4563A>G	hetero	snv
				c.3396A>G	hetero	snv
				c.-26G>A	hetero	snv
SUB 3	c.114G>A	hetero	snv	c.8187G>T	hetero	snv
	c.548-58delT	hetero	del	c.7397T>C	homo	snv
	c.2082C>T	hetero	snv	c.6513G>C	homo	snv
	c.2311T>C	hetero	snv	c.4563A>G	homo	snv
	c.2612C>T	hetero	snv	c.1114A>C	homo	snv
	c.3113A>G	hetero	snv			
	c.3548A>G	hetero	snv			
	c.4308T>C	hetero	snv			
	c.4485-63C>G	hetero	snv			
	c.4837A>G	hetero	snv			
SUB 4				c.7806-14T>C	hetero	snv
				c.7397T>C	homo	snv
				c.7242A>G	hetero	snv
				c.6841+80_6841+83delTTAA	hetero	del
				c.6513G>C	homo	snv
				c.4563A>G	homo	snv
				c.3807T>C	hetero	snv
				c.3396A>G	hetero	snv
				c.-26G>A	hetero	snv
SUB 5	c.548-58delT	homo	del	c.7397T>C	homo	snv
	c.2082C>T	homo	snv	c.6513G>C	homo	snv
	c.2311T>C	homo	snv	c.4563A>G	homo	snv
	c.2612C>T	homo	snv	c.1114A>C	homo	snv
	c.3113A>G	homo	snv			
	c.3548A>G	homo	snv			
	c.4308T>C	homo	snv			
	c.4485-63C>G	homo	snv			
	c.4837A>G	homo	snv			

SUBJECTS	BRCA1	Zygosity	Type	BRCA2	Zygosity	Type
SUB 6	c.548-58delT	hetero	del	c.7806-14T>C	hetero	snv
	c.2082C>T	hetero	snv	c.7397T>C	homo	snv
	c.2311T>C	hetero	snv	c.6513G>C	homo	snv
	c.2612C>T	hetero	snv	c.5199C>T	hetero	snv
	c.3113A>G	hetero	snv	c.4563A>G	homo	snv
	c.3548A>G	hetero	snv	c.1114A>C	hetero	snv
	c.4308T>C	hetero	snv			
	c.4485-63C>G	hetero	snv			
	c.4837A>G	hetero	snv			
SUB 7	c.114G>A	hetero	snv			
				c.7806-14T>C	hetero	snv
				c.7397T>C	homo	snv
				c.7242A>G	hetero	del
				c.6841+80_6841+83delTTAA	hetero	snv
				c.6513G>C	homo	snv
				c.4563A>G	homo	snv
				c.3396A>G	hetero	snv
				c.1114A>C	hetero	snv
				c.-26G>A	hetero	
SUB 8	c.114G>A	hetero	snv	c.7806-14T>C	hetero	snv
				c.7397T>C	homo	snv
				c.7242A>G	hetero	snv
				c.6841+80_6841+83delTTAA	hetero	del
				c.6513G>C	homo	snv
				c.4563A>G	homo	snv
				c.3396A>G	hetero	snv
				c.1114A>C	hetero	snv
				c.-26G>A	hetero	snv
SUB 9				c.7397T>C	homo	snv
				c.6513G>C	homo	snv
				c.4563A>G	homo	snv
				c.3807T>C	hetero	snv
				c.1114A>C	hetero	snv
SUB 10				c.7806-14T>C	homo	snv
				c.7397T>C	homo	snv
				c.7242A>G	hetero	snv
				c.6841+80_6841+83delTTAA	hetero	del
				c.6513G>C	homo	snv
				c.4563A>G	homo	snv
				c.3396A>G	hetero	snv


Syonymous variant
Intron variant
Missense variant
#Mutations
5
0
0
500
1000
1500
2000
2500
3000
3500
4000
4850aa
#Mutations
10
5
0
0
1000
2000
3000
4000
5000
6000
7000
8000
8200aa

Chromosome 17

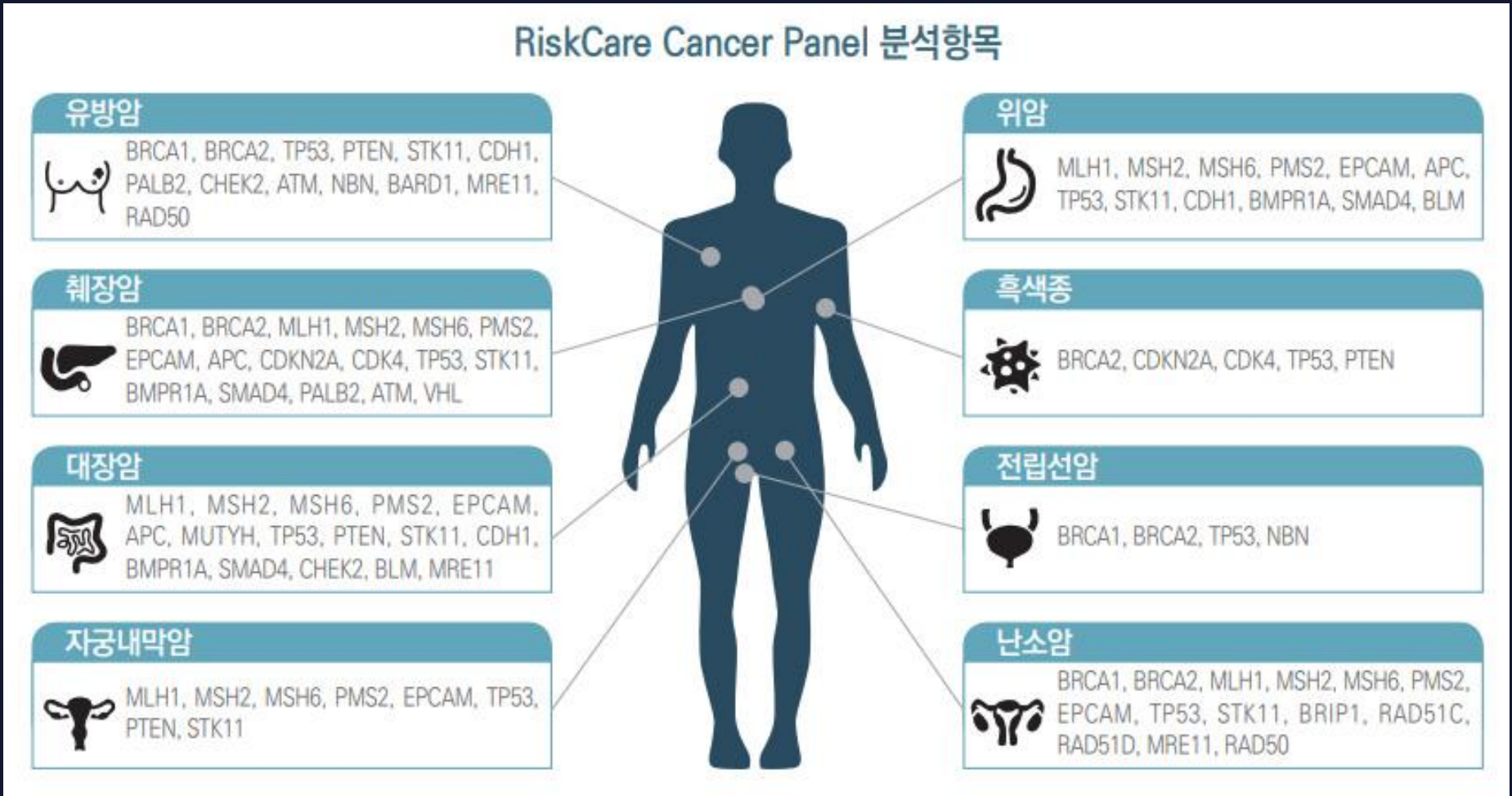
NT change (BIC Format)	AA change (Amino Acid)	Zygosity	Type	Classfication
c.4956A>G	p.Ser1613Gly	heterozygote	SNV	Benign
c.4604-182C>G		heterozygote	SNV	Benign
c.4427T>C	p.Ser1436=	heterozygote	SNV	Benign
c.3667A>G	p.Lys1183Arg	heterozygote	SNV	Benign
c.3232A>G	p.Glu1038Gly	heterozygote	SNV	Benign
c.2731C>T	p.Pro871Leu	heterozygote	SNV	Benign
c.2430T>C	p.Leu771=	heterozygote	SNV	Benign
c.2201C>T	p.Ser694=	heterozygote	SNV	Benign
c.667-177del		heterozygote	Del	Benign

Chromosome 13

NT change (BIC Format)`	AA change (Amino Acid)	Zygosity	Type	Classfication
c.1342A>C	p.Asn372His	heterozygote	SNV	Benign
c.4791A>G	p.Leu1521=	homozygote	SNV	Benign
c.5427C>T	p.Ser1733=	heterozygote	SNV	Benign
c.6741G>C	p.Val2171=	homozygote	SNV	Benign
c.7625C=	p.Ala2466=	homozygote	SNV	Benign
c.8034-242T>C		heterozygote	SNV	Benign

CONCLUSION

Currently, the NGS test, which can be performed in the diagnostic laboratory, can test as many as 100 tumor genes at once, and the entire gene or the area where mutations are concentrated can be selected and tested.



It is reported that the typical analytical sensitivity of the NGS test can detect mutations in the range of 1-10% of all bone marrow cells, which is known to exhibit more sensitive sensitivity than conventional sequencing methods. Therefore, the NGS test is expected to provide useful information for diagnosis, treatment determination, and follow-up of breast cancer and various carcinomas.

THANK YOU!